STATISTICAL ANALYSIS

Variability Associated with Sampling, Sample Preparation, and Chemical Testing for Aflatoxin in Farmers' Stock Peanuts

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Forty farmers' stock lots of runner peanuts suspected of containing aflatoxin were identified by the Federal State Inspection Service by using the visual *Aspergillus flavus* inspection method. A 900 kg portion was removed from each lot and divided into 50 samples each of 2.27 kg (5 lb), 4.54 kg (10 lb), and 6.81 kg (15 lb) weights. For each sample, foreign material was removed, pods were shelled, and all kernels were comminuted for 7 min in a vertical cutter mixer. A 100 g subsample was removed from each comminuted sample for aflatoxin analysis by liquid chromatography (LC). The total variance associated with each sample size was estimated. The total variance was also partitioned into sampling, sample preparation, and analytical variance components. Each variance component was shown to be a function of aflatoxin concentration. By using regression techniques, the relationship between variance and aflatoxin concentration was developed for each variance component. The total, sampling, sample preparation, and analytical variances associated with testing a lot at 100 ppb with a 2.27 kg sample, 100 g subsample, and using LC analytical techniques are 25 378, 23 533, 1830, and 15, respectively. Sampling, sample preparation, and analysis account for 92.7, 7.2, and 0.1% of the total variability, respectively.

All farmers' stock peanut lots marketed in the United States are presently inspected for the presence of the aflatoxin-producing fungus *Aspergillus flavus* during the grading operation at the buying point. Approximately 500 g of kernels from the grade sample are visually inspected for the presence of the fungus. If 1 or more kernels are found with the specific fungus, the farmer's lot is classified segregation III and diverted from the edible market (1). The visual inspection procedure is rapid and inexpensive and fits well into the buying-point environment. However, the visual inspection method does not measure aflatoxin directly, and the variability associated with the 500 g sample size is large (2–4).

With increased emphasis, both in the domestic and international markets, to reduce the levels of aflatoxin in food products, the U.S. peanut industry promoted studies to determine if the visual inspection method can be replaced with a chemical-testing program at the buying point. Two major projects were developed: (*1*) a time and motion or feasibility study to determine if the steps associated with a chemical-testing plan would fit into the grading operation at the buying point and (*2*) a variability study to determine the effects of sample size on the efficacy of detecting and classifying contaminated lots.

The variability study provides the basic information needed to develop a statistically based method to evaluate aflatoxin sampling plans for farmers' stock peanuts. The evaluation method is used to determine the effects of sample size and tolerance levels on such important attributes of an aflatoxin-testing plan as the farmer's risk (false positives), sheller's risk (false negatives), number of lots removed from the edible market, amount of aflatoxin removed from the crop, and total cost of a testing plan to the peanut industry. Designing an effective aflatoxin-testing plan is greatly enhanced by the development of a statistical evaluation method.

Whitaker et al. (5, 6) and Whitaker (7) measured the variability associated with testing shelled peanuts for aflatoxin and developed an evaluation method that is currently used by the peanut industry to design aflatoxin-testing plans for most

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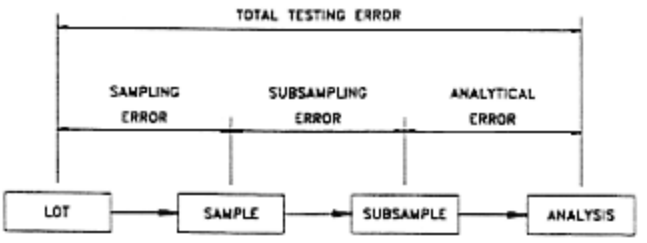

Figure 1. Typical steps in an aflatoxin-testing plan to estimate the aflatoxin concentration, $\bar{x}$, and the associated variance components.

shelled peanuts marketed in the United States. It is assumed that the variability associated with testing shelled peanuts for aflatoxin is different from that associated with testing farmers' stock peanuts for aflatoxin, because several peanut components that are correlated with the presence of aflatoxin (such as damaged and small peanuts) are removed from the farmer's lot during the shelling and cleaning process.

To facilitate development of a statistical evaluation method, the objectives of this study were (*1*) to determine the total variability associated with chemically testing farmers' stock peanuts for aflatoxin and (*2*) to partition the total variance into components that reflect sampling, sample preparation, and analytical variability.

Experimental

From the 1990 crop, 40 farmers' stock lots (averaging about 4100 kg or 9000 lb each) of runner peanuts suspected of containing aflatoxin were identified by the Federal State Inspection Service using the visual inspection method. A 900 kg (1982 lb) portion was removed from each of the 40 lots by using a divider as the peanuts were being unloaded. With a specially constructed divider, each 900 kg portion was divided into 64 samples each of 2.27 kg (5 lb), 4.54 kg (10 lb), and 6.81 kg (15 lb) weights. For each lot, 50 samples of each size, or a total of 6000 samples, were tested for aflatoxin in the variability study. Some of the remaining 14 samples per sample size per lot were used in other studies. From the excess samples, a 4.54 kg sample from each lot was used to estimate the number of pods per kilogram.

Each sample contained all components of a farmer's stock lot, foreign material, loose shelled kernels, and pods. The weight of each sample component (foreign material, loose shelled kernels, hulls, and shelled kernels) was recorded. Sample weights in this study excluded foreign material and reflected loose shelled kernels and pods only. For each sample, the foreign material was removed and the pods were shelled. All kernels (loose shelled kernels and shelled kernels were combined) were comminuted in a Stephan vertical cutter mixer (VCM) for 7 min. The sample preparation methods (type of mill and comminution time) were similar to those used in the feasibility study. A 100 g subsample, regardless of sample size, was removed from the comminuted sample. Aflatoxin was extracted from the 100 g subsample with acetonitrile–water (90 + 10, v/v) in a 3:1 solvent volume:peanut weight ratio. The extract was purified by using a Mycosep 224 column (Romer Labs, Inc., Washington, MO), and aflatoxins were quantified by reversed-phase liquid chromatography (LC) as described by Wilson and Romer (8) and Hagler and Whitaker (9). Aflatoxin concentrations are reported as parts per billion (ppb) and reflect the sum of aflatoxins B_1, B_2, G_1, and G_2.

Theoretical Considerations

We assumed that (*1*) the farmer's lot consists of N individual items and the individual item is a peanut pod, (*2*) each pod has the same mass, and (*3*) the aflatoxin concentration, x in ppb, varies from peanut pod to peanut pod. Usually, the concentration x is zero, but occasionally, x can be extremely high. Because it is not practical to measure the aflatoxin concentration in individual peanut pods, the standard practice is to draw a sample of np peanut pods from the lot and then determine the aflatoxin concentration of the whole sample. The aflatoxin concentration in the np peanut pods in the sample is denoted by $\bar{x}$ and is expressed in ppb. The sample concentration $\bar{x}$ is estimated by the test procedure outlined above.

The sources of the variability associated with obtaining an aflatoxin test result $\bar{x}$ are illustrated in Figure 1. As the figure illustrates, the total variance among aflatoxin test results can be composed of at least 3 variance components: sampling, subsampling, and analysis. A reasonable mathematical model for the observed aflatoxin test result, $\bar{x}$, can be represented as follows:

$$\bar{x} = \mu + S + SS + A \qquad (1)$$

where μ = the true aflatoxin concentration in the farmer's lot being tested, S = random deviations of sample concentrations about the lot concentration with expected value zero and variance $\sigma^2_{\bar{x}(s)}$, SS = random deviations of subsample concentrations about the comminuted sample concentration with expected value zero and variance $\sigma^2_{\bar{x}(ss)}$, and A = random deviations of analytical assay results about the subsample concentration with expected value zero and variance $\sigma^2_{\bar{x}(a)}$. By assuming independence among the random deviations in equation 1, the following variance relationship is obtained:

$$\sigma^2_{\bar{x}(t)} = \sigma^2_{\bar{x}(s)} + \sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)} \qquad (2)$$

where $\sigma^2_{\bar{x}(t)}$ = total variance associated with an aflatoxin test result $\bar{x}$.

The total variance and the analytical variance were estimated by direct measurement. The sampling variance and the subsampling variance were estimated indirectly by using the summation property shown in equation 2. Estimates of each variance component in equation 2 and the true lot aflatoxin concentration μ in equation 1 from experimental values are denoted by $s^2_{\bar{x}}$ and $\bar{\bar{x}}$, respectively. The notation $\bar{\bar{x}}$ is defined as the average aflatoxin concentration among the 50 sample aflatoxin test results $\bar{x}$.

Total variance.—The total variance, $\sigma^2_{\bar{x}(t)}$, is defined as the variance among aflatoxin test results on 50 samples (1 assay result per sample) of the same size taken from the same lot of farmers' stock peanuts. The estimated total variance $\sigma^2_{\bar{x}(t)}$ and the average aflatoxin concentration $\bar{\bar{x}}$ among the 50 sample test results for each of the 3 sample sizes were calculated for each of the 40 lots to provide 120 estimates of total variance: 40 estimates for the 2.27 kg (5 lb) sample size, 40 estimates for the 4.54 kg (10 lb) sample size, and 40 estimates for the 6.81 kg (15 lb) sample size.

Analytical variance.—The analytical variance, $\sigma^2_{\bar{x}(a)}$, represents the variability among aflatoxin determinations on equal aliquots of extract taken from the blender after the extraction step. Eighteen naturally contaminated samples of comminuted peanut kernels weighing 100 g each were blended 3 min with 300 mL acetonitrile–water. The peanut and solvent blend was divided into 19 equal volumes or aliquots, each aliquot was prepared for quantitation (8), and aflatoxin was quantified by reversed-phase LC (9). Nineteen replicated aflatoxin determinations were made on each of the 18 samples. The 18 samples were chosen with aflatoxin concentrations that varied from about 5 to 4000 ppb. All analytical tests were conducted in the same laboratory and reflect within-laboratory variability only. The estimated analytical variance, $\sigma^2_{\bar{x}(a)}$, and the average aflatoxin concentration $\bar{\bar{x}}$ among the 19 aflatoxin determinations were calculated for each of the 18 samples.

Sampling variance.—The sampling variance is defined as the variance among the aflatoxin concentration of replicate samples of the same size taken from the same lot. The sampling variance was estimated indirectly by using the summation properties for total variance shown in equation 2. The total variance associated with the 2.27 kg (5 lb) sample size, the 4.54 kg (10 lb) sample size, and the 6.81 kg (15 lb) sample size are shown in equations 3–5, respectively.

$$\sigma^2_{\bar{x}(t)_5} = \sigma^2_{\bar{x}(s)_5} + \sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)} \quad (3)$$

$$\sigma^2_{\bar{x}(t)_{10}} = \sigma^2_{\bar{x}(s)_{10}} + \sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)} \quad (4)$$

$$\sigma^2_{\bar{x}(t)_{15}} = \sigma^2_{\bar{x}(s)_{15}} + \sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)} \quad (5)$$

Because the subsampling and analytical procedures were the same for all tests, all subsampling variance terms, $\sigma^2_{\bar{x}(ss)}$, are equal and all analytical variance terms, $\sigma^2_{\bar{x}(a)}$, are equal in equations 3–5. Only the total variance and the sampling variance differ in equations 3–5 because of sample size.

Statistical theory (10) indicates that larger samples of peanuts will be less variable than smaller samples. It follows that sampling variances for a 2.27, 4.54, and 6.81 kg samples are related to each other by the following equations:

$$\sigma^2_{\bar{x}(s)_5} = \left(\frac{np_{15}}{np_5}\right) \times \sigma^2_{\bar{x}(s)_{15}} \quad (6)$$

Table 1. Average number of pods and average weight of samples used in the variability study

	Actual	
Targeted weight, kg	Number of pods	Weight, kg
2.27	1999	2.26
4.54	3724	4.21
6.81	6097	6.91

$$\sigma^2_{\bar{x}(s)_{10}} = \left(\frac{np_{15}}{np_{10}}\right) \times \sigma^2_{\bar{x}(s)_{15}} \quad (7)$$

$$\sigma^2_{\bar{x}(s)_5} = \left(\frac{np_{10}}{np_5}\right) \times \sigma^2_{\bar{x}(s)_{10}} \quad (8)$$

where np_5, np_{10}, and np_{15} are the average number of pods in the 50 samples each of 2.27, 4.54, and 6.81 kg weights, respectively. In equation 3, the sampling variance for a 2.27 kg sample can be written in terms of the sampling variance for a 6.81 kg sample by substituting equation 6 into equation 3.

$$\sigma^2_{\bar{x}(s)_5} = \left(\frac{np_{15}}{np_5}\right) \times \sigma^2_{\bar{x}(s)_{15}} + (\sigma^2_{\bar{x}(ss)} + \sigma^2_{\bar{x}(a)}) \quad (9)$$

By subtracting equation 5 from equation 9, the sampling variance for a 6.81 kg sample (equation 10) can be estimated:

$$\sigma^2_{\bar{x}(s)_{15}} = \frac{(\sigma^2_{\bar{x}(t)_5} - \sigma^2_{\bar{x}(t)_{15}})}{\left(\frac{np_{15}}{np_5}\right) - 1} \quad (10)$$

A second estimate of the sampling variance for a 6.81 kg sample can be obtained similarly by substituting equation 7 into equation 4 and subtracting equation 5:

$$\sigma^2_{\bar{x}(ss)_{15}} = \frac{(\sigma^2_{\bar{x}(t)_{10}} - \sigma^2_{\bar{x}(t)_{15}})}{\left(\frac{np_{15}}{np_{10}} - 1\right)} \quad (11)$$

From equations 10 and 11, up to 80 estimates of the sampling variance for a 6.81 kg sample $\sigma^2_{\bar{x}(ss)_{15}}$ can be obtained by using all 120 estimates of the total variance for the 3 sample sizes. Estimates of the sampling variance for a 2.27 kg and a 4.54 kg sample can be computed by using the relationships in equations 6 and 7, respectively.

Subsampling variance.—The subsampling variance, $\sigma^2_{\bar{x}(ss)}$, is defined as the variance among the aflatoxin concentrations of replicate 100 g subsamples taken from the same sample of peanuts comminuted in a Stephan VCM for 7 min. Estimates of the subsampling variance can be determined indirectly by using equations 3–5. Having developed estimates of total, sam-

Table 2. Number of replicate 2.26 kg samples, average sample size in number of pods, average aflatoxin concentration, total variance, and coefficient of variation among replicate sample aflatoxin test results for each lot[a]

Lot number	Number of samples	Number of pods	Aflatoxin concentration, ppb	Total variance	Coefficient of variation, %
27	50	1829.7	33.8	11010.3	310.1
39	50	2006.0	79.0	13362.9	146.3
32	50	2623.1	83.2	9664.2	118.1
28	50	1678.7	106.3	35079.7	176.3
18	50	2011.8	106.5	28843.9	159.5
6	50	1903.6	129.2	42894.7	160.3
19	51	1785.8	173.9	50399.9	129.1
30	50	1747.5	186.4	35669.0	101.3
36	50	1942.0	215.2	33892.2	85.6
29	50	1724.9	262.0	81828.3	109.2
35	50	2161.1	275.8	26757.8	59.3
20	50	2757.8	352.3	48676.1	62.6
24	50	1855.2	358.2	109329.6	92.3
34	50	1863.6	431.6	117180.0	79.3
17	50	2107.8	514.7	165450.8	79.0
1	52	1836.4	547.3	82529.3	52.5
33	50	2453.8	567.3	76884.5	48.9
38	50	2186.5	599.3	176686.6	70.1
40	50	2008.7	691.8	150767.7	56.1
13	53	2123.7	843.7	158133.7	47.1
21	50	1947.4	906.5	206285.1	50.1
26	50	2286.2	945.7	172424.4	43.9
12	50	1826.0	955.2	219437.4	49.0
2	50	1790.5	1031.8	163073.8	39.1
22	50	1575.8	1072.6	436925.9	61.6
9	50	2150.9	1092.4	201885.7	41.1
37	50	2381.3	1114.8	307662.7	49.8
8	53	1900.6	1126.9	243447.9	43.8
23	50	2059.4	1181.7	470343.7	58.0
16	50	1767.1	1240.0	304858.6	44.5
31	50	2411.1	1753.4	340575.7	33.3
14	49	1826.7	1768.3	381287.1	34.9
5	50	1683.6	2025.7	546171.8	36.5
3	58	2136.9	2548.2	974489.0	38.7
25	50	1891.0	2697.9	995046.4	37.0
15	50	1981.1	3034.5	763110.3	28.8
11	50	2008.8	4656.3	992809.7	21.4
4	54	2017.4	7079.5	2100345.0	20.5
7	50	1898.1	8124.8	6677497.0	31.8
10	50	1820.3	24876.7	7833388.0	11.3

[a] Results are given in the order of increasing average aflatoxin concentration.

pling, and analytical variances, the only unknown in equations 3–5 is the subsampling variance term, $\sigma^2_{\bar{x}(ss)}$:

$$\sigma^2_{\bar{x}(ss)} = \sigma^2_{\bar{x}(t)_5} - \sigma^2_{\bar{x}(s)_5} - \sigma^2_{\bar{x}(a)} \quad (12)$$

$$\sigma^2_{\bar{x}(ss)} = \sigma^2_{\bar{x}(t)_{10}} - \sigma^2_{\bar{x}(s)_{10}} - \sigma^2_{\bar{x}(a)} \quad (13)$$

$$\sigma^2_{\bar{x}(ss)} = \sigma^2_{\bar{x}(t)_{15}} - \sigma^2_{\bar{x}(s)_{15}} - \sigma^2_{\bar{x}(a)} \quad (14)$$

From equations 12–14, up to 120 estimates of the subsampling variance can be computed.

Results and Discussion

The average sample size in kilograms and the average number of pods for the 3 sample sizes used in the study are shown

Table 3. Number of replicate 4.21 kg samples, average sample size in number of pods, average aflatoxin concentration, total variance, and coefficient of variation among replicate sample aflatoxin test results for each lot[a]

Lot number	Number of samples	Number of pods	Aflatoxin concentration, ppb	Total variance	Coefficient of variation, %
27	50	3325.7	31.6	4577.7	213.9
28	50	3334.5	46.1	6584.9	176.1
32	50	4989.0	50.2	2483.7	99.3
18	50	4080.3	64.1	5046.2	110.8
39	50	3383.4	100.5	14572.2	120.1
6	50	3396.2	124.5	15271.0	99.3
30	50	3120.5	158.2	21631.8	93.0
36	50	3417.8	225.9	24424.2	69.2
29	50	3382.6	236.3	37630.3	82.1
19	50	3579.0	250.5	11204.1	42.3
20	50	4783.0	253.7	17685.1	52.4
24	50	3477.0	295.1	30767.8	59.4
35	50	3775.6	318.6	28142.0	52.6
34	49	3243.6	432.7	82654.3	66.4
17	50	3772.8	502.5	59785.6	48.7
33	50	4281.0	582.8	56044.3	40.6
38	50	3870.4	594.1	53199.0	38.8
1	51	3668.1	651.3	84888.0	44.7
40	50	3496.3	715.7	114915.2	47.4
9	51	4278.9	893.7	53474.6	25.9
21	50	3355.0	961.8	158787.5	41.4
26	50	3974.4	982.5	149787.2	39.4
22	49	3127.7	985.6	397823.2	64.0
37	50	4093.4	1039.1	153766.6	37.7
12	50	2979.2	1056.0	197515.3	42.1
13	50	4330.8	1067.7	121884.7	32.7
16	50	3624.6	1178.8	199861.9	37.9
2	50	3383.3	1204.7	139221.1	31.0
23	50	3875.1	1271.2	268238.6	40.7
8	51	3866.7	1325.6	352967.6	44.8
31	50	4602.6	1769.3	319269.8	31.9
14	50	3406.3	1841.8	297520.5	29.6
5	50	3340.5	2195.1	493512.7	32.0
25	50	3524.4	2469.1	382921.3	25.1
15	50	3763.3	3018.5	353869.5	19.7
3	54	4148.2	3093.3	725203.7	27.5
11	50	4062.2	4039.7	1281121.3	28.0
4	54	3937.2	7110.7	2345137.9	21.5
7	49	3665.2	7683.1	9818263.5	38.9
10	50	3245.7	29053.8	7670896.2	9.5

[a] Results are given in the order of increasing average aflatoxin concentration.

in Table 1. The actual average sample sizes closely matched the desired targets of 2.27, 4.54, and 6.81 kg and will be referred to as 5, 10, and 15 lb samples.

Total variance.—Tables 2–4 show for each lot the number of replicated samples, average sample size, average aflatoxin concentration, and total variability as depicted by the variance and the coefficient of variation for the 5, 10, and 15 lb sample sizes, respectively. In each table, the results are ordered by increasing average aflatoxin concentration. The average total aflatoxin concentration in the 40 lots varied from about 25 to 25 000 ppb. Several important characteristics are associated with the results shown in Tables 2–4. First, regardless of sample size, the magnitude of the total variance is larger than the aflatoxin concentration in the lot. Second, the total variance increases with aflatoxin concentration. Third, for a given lot, the total variance is usually highest for the 5 lb sample size and lowest for the 15 lb sample size. Fourth, for a given lot, the average aflatoxin concentration among the 50 samples is about the same for the 3 sample sizes. The first 2 observations are consistent with total variance data collected on shelled peanuts,

Table 4. Number of replicate 6.91 kg samples, average sample size in number of pods, average aflatoxin concentration, total variance, and coefficient of variation among replicate sample aflatoxin test results for each lot[a]

Lot number	Number of samples	Number of pods	Aflatoxin concentration, ppb	Total variance	Coefficient of variation, %
27	50	5652.3	23.7	1274.5	150.8
28	50	5358.1	44.4	2663.1	116.2
32	50	8176.2	67.6	2950.2	80.3
18	50	6540.3	84.6	7143.6	99.9
39	50	5768.6	87.4	8371.8	104.6
6	50	5592.0	145.4	24613.0	107.9
19	50	5683.1	160.3	16404.5	79.9
30	50	5257.6	187.7	23877.9	82.3
29	50	5416.6	208.8	54552.8	111.9
36	50	5793.8	250.8	18076.6	53.6
20	50	7538.6	308.8	17758.8	43.2
24	50	5632.2	316.4	18310.3	42.8
35	49	6351.0	323.3	34583.7	57.5
34	50	5534.0	417.5	50775.5	54.0
17	50	6334.2	511.3	40564.8	39.4
33	50	7170.8	574.0	66820.1	45.0
1	50	6073.9	633.9	35166.9	29.6
38	50	6475.8	647.4	66698.6	39.9
40	49	6024.9	734.2	56456.2	32.4
9	50	7057.2	813.1	39384.2	24.4
13	48	6693.7	925.7	56284.9	25.6
26	50	6727.4	934.9	77549.6	29.8
22	50	5011.1	936.6	271156.6	55.6
21	50	5655.9	994.4	88555.0	29.9
12	50	5107.7	1068.8	167471.9	38.3
23	50	6329.2	1143.3	104100.1	28.2
37	50	6861.2	1192.4	291768.9	45.3
16	50	5631.4	1309.0	133951.1	28.0
2	50	5334.7	1345.5	80460.9	21.1
8	54	6261.6	1475.2	100466.6	21.5
31	50	7587.6	1590.7	132218.6	22.9
5	54	5406.3	1733.4	110498.2	19.2
14	50	5631.8	1792.6	265032.2	28.7
3	56	6664.3	2606.6	268122.9	19.9
25	50	5815.3	3180.1	587598.1	24.1
15	50	6154.5	3330.0	457256.1	20.3
11	50	6557.4	4353.7	489685.0	16.1
4	55	5940.0	6972.2	1962791.0	20.1
7	48	5832.6	8215.1	13163362.0	44.2
10	50	5282.4	20453.2	9102229.0	14.8

[a] Results are given in the order of increasing average aflatoxin concentration.

shelled corn, and cottonseed (6, 11, 12). The last 2 observations are in agreement with statistical theory.

Figures 2–4 show a full log plot of total variance versus aflatoxin concentration for the 5, 10, and 15 lb sample sizes, respectively. The plots indicate that the total variance is a linear function of aflatoxin concentration in the full log scale. Therefore, it was assumed that a power function of the general form

$$\sigma_{\bar{x}}^2 = C1\,\mu^{C2} \qquad (15)$$

where C1 and C2 are constants, describes the empirical relationship between variance and aflatoxin concentration. By using the Statistical Analysis System (13), the constants C1 and C2 were determined by a least-squares fit of $\log_e(s^2_{\bar{x}(i)})$ to $\log_e(\bar{x})$.

From the regression analysis, the values of C2 (equation 15) for the 5, 10, and 15 lb variance data were 1.0912, 1.2406, and 1.2504, respectively. The 3 C2 values were similar, and the Studentized Range test (10) indicated that the null hypothesis, H_0 1.09 = 1.24 (maximum difference), cannot be rejected at the 5% significance level. Therefore, equation 15 was regressed on all

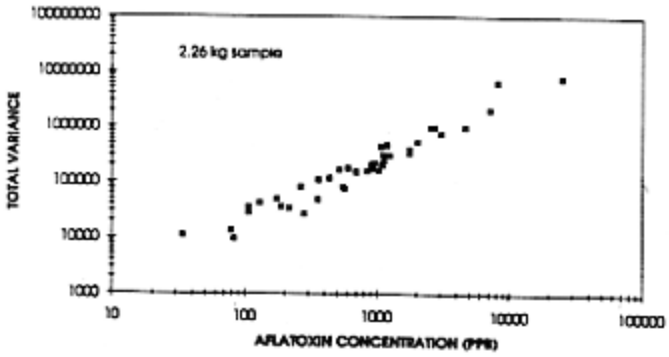

Figure 2. Total variance versus aflatoxin concentration for 2.26 kg sample pods.

120 total variance observations where the constant C2 (representing the slope) was held fixed across all sample sizes and the constant C1 was allowed to vary with sample size. The regression analysis gave the following expressions:

$$s^2_{\bar{x}(0)_5} = 66.0413\ (\bar{\bar{x}}\ )^{1.1976} \tag{16}$$

$$s^2_{\bar{x}(0)_{10}} = 40.8469\ (\bar{\bar{x}}\ )^{1.1976} \tag{17}$$

$$s^2_{\bar{x}(0)_{15}} = 30.1047\ (\bar{\bar{x}}\ )^{1.1976} \tag{18}$$

with a coefficient of determination of 0.93 in the log scale. The standard error of estimate for the (log C1) and C2 values are 0.225 and 0.032, respectively.

It can be seen in equation 2 that if the subsampling and analytical variances were approximately zero, the total variance would equal the sampling variance. Statistical theory predicts that the ratios of sampling variances for the 3 sample sizes should be

$$\frac{\sigma^2_{\bar{x}(s)_5}}{\sigma^2_{\bar{x}(s)_{10}}} = \frac{np_{10}}{np_5} = 1.86 \tag{19}$$

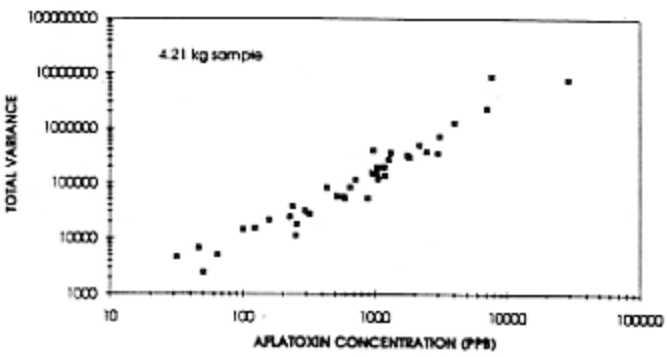

Figure 3. Total variance versus aflatoxin concentration for 4.21 kg sample pods.

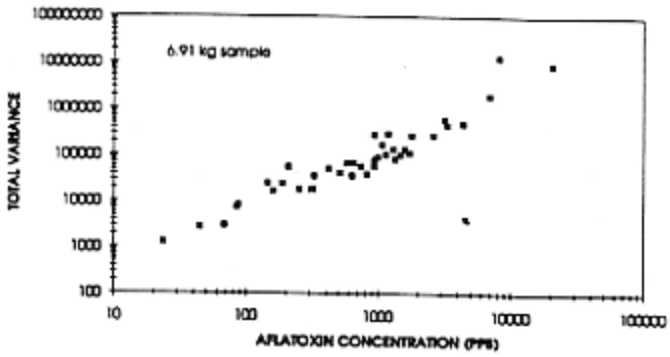

Figure 4. Total variance versus aflatoxin concentration for 6.91 kg sample pods.

$$\frac{\sigma^2_{\bar{x}(s)_5}}{\sigma^2_{\bar{x}(s)_{15}}} = \frac{np_{15}}{np_5} = 3.06 \tag{20}$$

$$\frac{\sigma^2_{\bar{x}(s)_{10}}}{\sigma^2_{\bar{x}(s)_{15}}} = \frac{np_{15}}{np_{10}} = 1.64 \tag{21}$$

The 3 ratios of total variances computed from equations 16–18 are

$$\frac{s^2_{\bar{x}(t)_5}}{s^2_{\bar{x}(t)_{10}}} = 1.62 \tag{22}$$

$$\frac{s^2_{\bar{x}(t)_5}}{s^2_{\bar{x}(t)_{15}}} = 2.19 \tag{23}$$

$$\frac{s^2_{\bar{x}(t)_{10}}}{s^2_{\bar{x}(t)_{15}}} = 1.36 \tag{24}$$

As will be shown later, the subsampling and analytical variances are greater than zero. Therefore, the experimentally determined variance ratios in equations 22–24 should be less than those predicted by equations 19–21.

Analytical variance.—The analytical variance, $s^2_{\bar{x}(s)}$, and the average aflatoxin concentration among the 19 replicate test results for each of the 18 samples are shown in Table 5. The analytical variances are ranked in the table by the aflatoxin concentration $\bar{\bar{x}}$. The aflatoxin concentration in the 18 samples varied from 0 to 3905 ppb. A full log plot of the analytical variance versus aflatoxin concentration is shown in Figure 5. As with total variance, the analytical variance increases with aflatoxin concentration and appears to be a linear function in the log scale. Regression equation 15 was fitted to the data in Table 5 to give:

$$s^2_{\bar{x}(a)} = 0.004828\,\bar{\bar{x}}^{1.7518} \quad (25)$$

with a coefficient of determination of 0.95 in the log scale. The standard error of estimate for (log C1) and C2 are 0.523 and 0.104, respectively.

The analytical variabilities associated with extraction, cleanup, and LC quantitation procedures are relatively small and reflect only within-laboratory variability. The coefficient of variation (CV) at total aflatoxin concentration of 20 ppb, computed from equation 25, is 4.8%. By comparison, the CV associated with other analytical procedures for peanuts that use thin-layer chromatography for quantitation is about 25% (14).

Sampling variance.—As shown in equations 10 and 11, up to 80 estimates of the sampling variance for a 6.81 kg sample $s^2_{\bar{x}(s)_{15}}$ can be obtained by subtracting the appropriate total variances shown in Tables 2–4 for each lot. Of the 80 possible estimates of sampling variance, 62 estimates were computed. The loss of 18 sampling variance estimates occurred when differences between the total variances for a given lot were negative. These negative variance estimates were not used. Negative values were obtained for 3 lots when the total variance among the 5 lb samples was smaller than the total variance among the 15 lb samples (see Tables 2 and 4). Negative values were obtained for 15 lots when the total variances among 10 and 15 lb samples were subtracted. A full log plot of $s^2_{\bar{x}(s)_{15}}$ versus aflatoxin concentration is shown in Figure 6. As with other variance components, the sampling variance increases with aflatoxin concentration and appears to be a linear function in the full log plot. Regression equation 15 was fit to the sampling variance data using log values. The regression analysis gave

$$s^2_{\bar{x}(s)_{15}} = 95.3565\,\bar{\bar{x}}^{0.9576} \quad (26)$$

with a coefficient of determination of 0.70 in the log scale. The standard error of estimate for (log C1) and C2 are 0.541 and 0.0817, respectively. By use of equations 6 and 7, the sampling variances for a 4.54 and a 2.27 kg sample become, respectively.

$$s^2_{\bar{x}(s)_{10}} = \left(\frac{np_{15}}{np_{10}}\right)95.3565\,\bar{\bar{x}}^{0.9576} \quad (27)$$

$$s^2_{\bar{x}(s)_5} = \left(\frac{np_{15}}{np_5}\right)95.3565\,\bar{\bar{x}}^{0.9576} \quad (28)$$

Values of np_5, np_{10}, and np_{15} can be found in Table 1.

Subsampling variance.—By using equations 12–14, up to 120 estimates of the subsampling variance can be obtained. In Tables 2–4, a sampling and analytical variance component was subtracted from the total variance for each lot. Equation 25 was used to estimate the analytical variance component, and equations 26–28 were used to estimate the sampling variance com-

Table 5. Average aflatoxin concentration, analytical variance, and coefficient of variation among replicate aflatoxin test results on 19 aliquots quantified by LC procedures[a]

Subsample number	Aflatoxin concentration, ppb	Analytical variance	Coefficient of variation, %
1	0.0	0.00	0.00
2	5.7	0.32	9.92
3	14.5	0.74	5.93
4	17.3	0.33	3.32
5	28.5	1.63	4.48
6	31.9	4.02	6.29
7	65.4	7.29	4.13
8	70.9	3.43	2.61
9	75.6	4.46	2.79
10	87.1	3.04	2.00
11	129.0	44.58	5.18
12	129.3	35.00	4.58
13	250.8	47.19	2.74
14	399.8	331.16	4.55
15	611.7	336.44	3.00
16	802.5	432.16	2.59
17	1018.4	1992.13	4.38
18	3905.4	11582.37	2.76

[a] LC procedures are from references 8 and 9. All assays were conducted within a single laboratory.

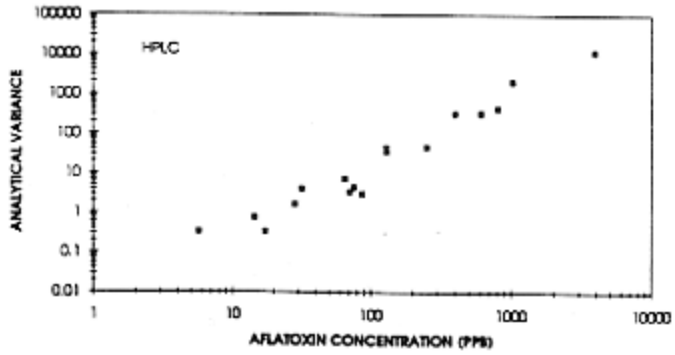

Figure 5. Analytical variance versus aflatoxin concentration for the Wilson-Romer extraction and cleanup procedure with LC quantitation.

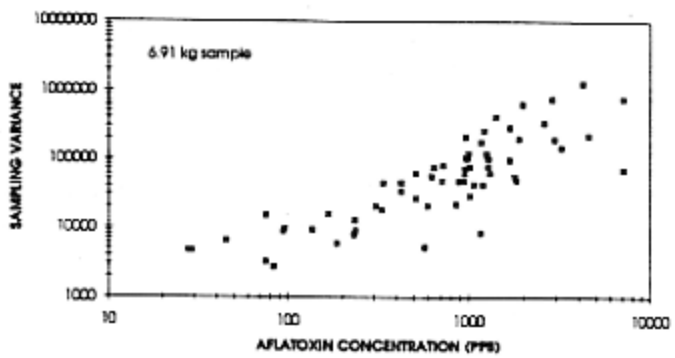

Figure 6. Sampling variance versus aflatoxin concentration for 6.91 kg sample pods.

ponents. Of the possible 120 subsampling variances estimates, 39 estimates were negative, leaving 81 subsampling variance estimates for analysis. A full log plot of the 81 subsampling variance estimates versus aflatoxin concentration is shown in Figure 7. As with the other variance components, the subsampling variance increases with aflatoxin concentration and appears to be a linear function in the full log plot. Therefore, regression equation 15 was fit to the subsampling variance data, using log values. The regression analysis gave

$$s^2_{\bar{x}(ss)} = 2.8886\,\bar{\bar{x}}^{1.4009} \qquad (29)$$

with a coefficient of determination of 0.68 in the log scale. The standard error of estimate for (log C1) and C2 are 0.754 and 0.108, respectively.

The total variance associated with the aflatoxin test procedure used in this study can be reduced by reducing 1 or more of the variance components in equation 2. One way is to increase the quantity of material inspected. The sampling variance can be reduced by increasing sample size, the subsampling variance can be reduced by increasing the subsample size, and the analytical variance can be reduced by increasing the number of aliquots taken from the blender in the extraction step and quantified by LC. The sampling variance in equation 26 can be modified to predict the effect of any size sample, *ns*, on the sampling variance.

$$s^2_{\bar{x}(s)_{ns}} = \left(\frac{658.9}{ns}\right)\bar{\bar{x}}^{0.9576} \qquad (30)$$

where *ns* is the sample size in kilograms. Equation 30 can also be written for sample size in number of pods, *np*, but it may be easier to calculate sampling variance based on weight.

A similar expression exists for the subsampling variance described by equation 29. The subsampling variance for any size subsample, *nss*, where the sample has been comminuted in a VCM becomes

$$s^2_{\bar{x}(ss)_{nss}} = \left(\frac{288.86}{nss}\right)\bar{\bar{x}}^{1.4009} \qquad (31)$$

where *nss* is the subsample size in grams.

The analytical variance, described by equation 25 for the analysis of a single aliquot quantified by LC, can be computed for any number of aliquots, *na*.

$$s^2_{\bar{x}(a)_{na}} = \left(\frac{0.004828}{na}\right)\bar{\bar{x}}^{1.7518} \qquad (32)$$

By adding equations 30–32, the total variance can be estimated for any size sample, any size subsample, and any number of aliquots quantified by LC:

$$s^2_{\bar{x}(t)} = \left(\frac{658.9}{ns}\right)\bar{\bar{x}}^{0.9576} + \left(\frac{288.86}{nss}\right)\bar{\bar{x}}^{1.4009} + \left(\frac{0.004828}{na}\right)\bar{\bar{x}}^{1.7518} \qquad (33)$$

Equation 33 was used to predict the total variance for the 3 sample sizes used in this study. The predicted total variances for 2.26, 4.21, and 6.91 kg sample sizes are plotted against the measured total variances in Figures 8–10, respectively. The previous equations developed to predict total variance (equations 16–18) are unique for the conditions of this study and do not have the flexibility of equation 33 to predict the total variance for any size sample, any size subsample, and any number of aliquots used in a chemical-testing program.

From equation 33, the total variance associated with testing a farmer's peanut lot contaminated with aflatoxin at 100 ppb when using a 2.27 kg sample, a VCM to comminute the sample for 7 min, a 100 g subsample of comminuted peanuts, and LC quantitation procedures is 25 378.3 (CV = 159.6%). The sampling, subsampling, and analytical variances are 23 532.7, 1830.2, and 15.4, respectively, and account for 92.7, 7.2, and 0.1% of the total variation, respectively. If sample size is increased to 40 kg, aflatoxin is extracted from a 200 g subsample, and 1 aliquot is quantified, the total, sampling, subsampling, and analytical variances associated with testing a lot at 100 ppb are 2266.0, 1335.5, 915.1, and 15.4, respectively; sampling, subsampling, and analytical variances account for 58.9, 40.4, and 0.7% of the total variation, respectively. A total variance of 2266.0 suggests that if aflatoxin test results are normally dis-

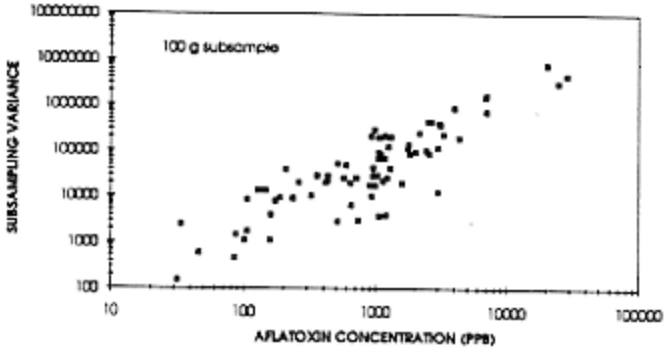

Figure 7. Subsampling variance versus aflatoxin concentration for 100 g subsamples comminuted in a Stephan vertical cutter mixer for 7 min.

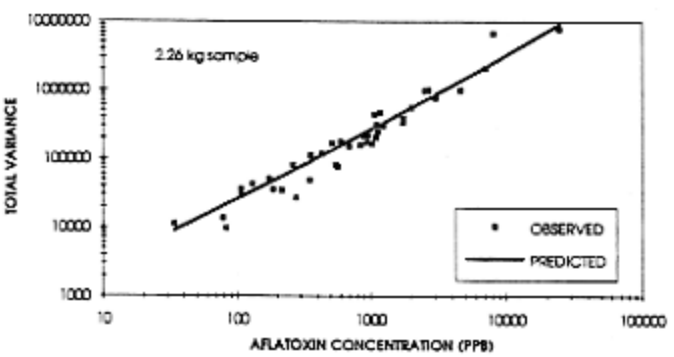

Figure 8. Measured and predicted total variances versus aflatoxin concentration for 2.26 kg sample of pods.

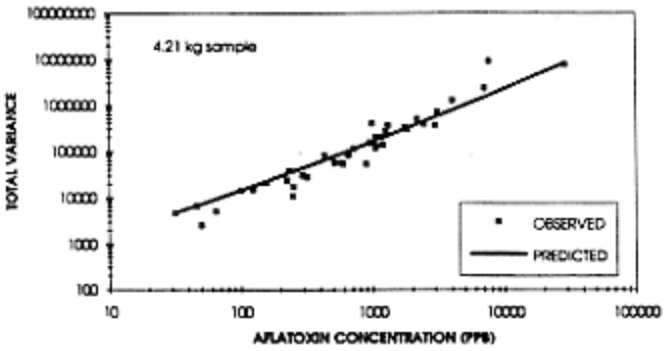

Figure 9. Measured and predicted total variances versus aflatoxin concentration for 4.21 kg sample of pods.

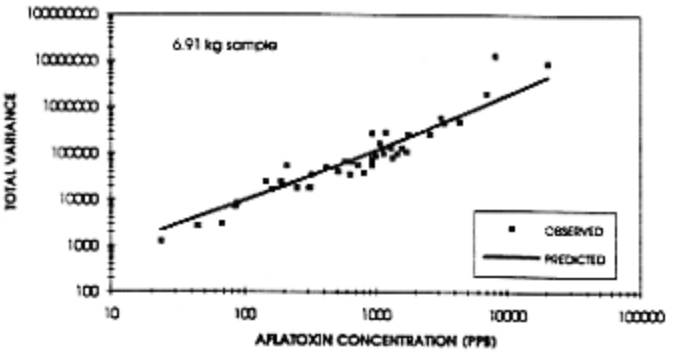

Figure 10. Measured and predicted total variances versus aflatoxin concentration for 6.91 kg sample of pods.

tributed (which is true only for large sample sizes), aflatoxin test results for the above test procedures will fall in the range of 100 ± 93 ppb, or 7–193 ppb, 95% of the time.

The total variances measured in this study reflect sampling farmers' stock peanuts, a 100 g subsample comminuted in a VCM for 7 min, extraction and cleanup procedures described by Wilson and Romer (8), and LC quantitation methods described by Hagler and Whitaker (9). It is not clear yet what sample preparation and analytical methods will be used in a regulatory environment if farmers' stock peanut are analyzed chemically for aflatoxin. Once the Agricultural Marketing Service and the peanut industry select sample preparation and analytical methods for a regulatory program, the total variance estimates can be adjusted by replacing variance equations 31 and 32 with appropriate variance equations describing the specific sample preparation and analytical methods. However, if small sample sizes are used in a future testing program, the total variance will not change significantly with different sample preparation and analytical methods, because the sampling variance accounts for most of the total variance (92.7% for a 2.27 kg sample).

Because the experimentally determined variance components appear to be functionally related to the aflatoxin concentration, the assumption made concerning the independence of the random errors S, SS, and A in equation 1 may be open to question. Other statistical models, such as a multiplicative model, were investigated but did not provided a workable alternative. However, the variance relationships presented in this study indicate the major sources of variation when testing farmers' stock peanuts for aflatoxin and indicate approaches to effectively reduce the total variability.

Future studies are required to develop a statistical evaluation method to predict the effects of sample size and tolerance levels on the accuracy of classifying a farmer's lots according to their aflatoxin concentration. Theoretical distributions will be compared with the 120 observed distributions of aflatoxin test results shown in this study to determine a model that will accurately predict the probability of accepting a farmer's lot for any aflatoxin sampling design and tolerance level.

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